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O-4 Tα1 α-Tubulin Promoter Directs Neuron-Specific Expression of Green Fluorescent Protein in Loach Embryo

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INTRODUCTION

Studies on promoter function or lineage analysis require the expression of foreign reporter gene, such as the bacterial gene lacZ, to identify tissue harboring a transgene. Typically, transgene expression has been identified by in situ hybridization or by histochemistry in fixed tissue. However, the inability of detecting transgene expression in living animals limits the utility of this technology, particularly in dynamic expression analysis. Utilization of green fluorescent protein(GFP) as a reporter gene overcomes this limitation and enables transgene expression to be observed in living embryos. The GFP, a single peptide of 238 amino acids derived from the jellyfish *Aequorea victoria,* absorbs blue light and emits green light without the need for any cofactor or substrate.

Fish has been considered to be an attractive system for the study of gene expression or cell-lineage during vertebrate development due to easy accessibility and transparent feature of its embryos. Other advantages of using the fish include rapid embryonic development and large number of progeny from a single mating. In this study, we used loach(*Misgurnus mizolepis)* embryos to investigate the activity of neuron-specific promoter during development. Rat Tα1 α-tubulin gene is abundantly expressed in developing rat neurons during morphological growth and in regenerating mature neurons. It has been demonstrated that 1.1kb 5'-flanking region of rat Tα1 α-tubulin gene was capable of directing developing neuron-specific expression in transgenic mice(A.Gloster, 1994).

Aims of this study are two-fold : to demonstrate that the GFP under control of Tα1 α-tubulin promoter is reliable reporter for certain subset of developing neurons in loach embryo, and to examine whether this system could allow investigation of dynamic changes in gene expression and morphology in developing neurons as a vital marker.

MATERIALS & METHODS

Female loach was superovulated with an intraperitoneal injection of human chorionic gonadotropin(hCG). The oocytes were obtained from abdomen and utilized in artificial fertilization. To obtain promoter region, 1.1kb 5'-flanking sequences of the Tα1 α-tubulin gene were amplified by PCR using rat genomic DNA as template. CMV promoter sequence was removed form pEGFP-C1, a GFP expression vector, by Ase I /Age I double digestion. pNTGFP was constructed by ligating PCR product with promoter-less pEGFP-c1. pNTGFP was linealized by ApaLI digestion and microinjected into 1-cell fertilized eggs of wild-type loach at concentration of 50μg/ml. Injection volume was adjusted to 4.5 nl per embryo using motorized microinjector. Embryos were examined under fluorescent microscope at various developmental stages.

RESULTS & DISCUSSION

GFP reporter gene expression was examined during throughout embryonic developmen. It was also investigated if Tα1 α-tubulin promoter could show neuron-specificity in developing loach embryos. GFP expression was observed as early as 50% epiboly stage, approximately 6h postfertilization(pf) at 28℃, although expression levels varied even in embryos injected with same amount of DNA. During the gastrulation, GFP expression was mainly visible in the dorsal animal pole fated to notochord and brain. As the neurulation and somitogenesis proceed the expression of GFP was gradually restricted to neural cells. At the pre-hatching and fry stages, GFP expression was clearly visible in neurons including extended axons. Expression intensity was high in the brain and eyes. In the peripheral nervous system, GFP expression was detected in primary sensory neurons of dorsal root ganglion. However, only weak GFP expression was shown in the spinal cord. Although GFP-expression was detectable in non-neural cells, its neuronal

specificity was comparably high(Table 1) in embryos with various expression intensity.

Non-neuron specific expression were confined to muscle cells and yolk syncytial layer. This may be due to a lack of silencing transcription factors that recognize neural restricted silencer element in the rat Tα1 α-tubulin promoter. After 7days pf, GFP positive cells was nearly unrecognizable. Previously, It has been demonstrated that Tα1 α-tubulin mRNA levels decrease coincidently with neuronal maturation, which may be the explanation for extinction of GFP expression in loach fry. However, the possibility of degradation of transgene in fry can not be excluded.

Our results showed that rat promoter was properly recognized in loach embryos by the corresponding transcription factors. Thus, the rat Tα1 α-tubulin promoter can be utilized for neuron-specific reporter gene expression and may facilitate the spatio-temporal analysis of the dynamic process of neuronal development in fish embryos.

Table 1. Transient expression of GFP in embryos microinjected with pNTGFP

No. of microinjected eggs	No. of fry survived at 48h pf(%)	No. of GFP-positive embryos(%)	No. of fry with neuron-specific GFP expresseion(%)	No. of fry with Non-neuronal GFP expression(%)
155	122(79)	86(70)	61(71)*	25(29)†

*: Embryos with GFP expression mainly in neurons.
†: Embryos with GFP expression mainly in non-neuronal tissues such as muscle and yolk syncytium.

SUMMARY

A DNA construct containing rat Tα1 α-tubulin gene 5'-flanking sequence and GFP reporter gene was microinjected into 1-cell loach embryos. Neuron-specific GFP expression was observed in developing loach embryos and early stage fry. The results demonstrated that rat Tα1 α-tubulin gene promoter may be sufficient to specify gene expression to neurons in loach embryos. Thus, the use of GFP reporter controlled by Tα1 α-tubulin gene promoter may facilitate visualization of the dynamic processes of neural tissue development.

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